

[0016] In addition, noise can be reduced to enhance S/N ratio by adjusting polarized lights to excite or suppress some specific vibrating or rotating modalities.

[0017] Also, the light detector is preferably designed to detect the SPR and light interference of TM and TE light waves simultaneously for filtering out the background noise effectively to improve accuracy of detection.

[0018] For more detailed information regarding advantages and features of the present invention, examples of preferred embodiments will be described below with reference to the annexed drawings.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

[0019] The related drawings in connection with the detailed description of the present invention to be made later are described briefly as follows, in which:

[0020] Figure 1 is a schematic view showing a surface plasmon resonance (SPR) sensor with high sensitivity in accordance with the present invention;

[0021] Figure 2 is a schematic view showing a SPR sensor of the present invention;

[0022] Figure 3 shows the enhanced Raman scattering spectra of metallic nanoparticle layers;

[0023] Figure 4 shows spectral curves of various structures of film layers;

[0024] Figure 5 shows data comparison of detected gases made by a conventional SPR sensor and the present invention;

[0025] Figure 6 is a schematic view of a biosensor based on the layer structure of the present invention;

[0026] Figure 7A shows detection of DNA hybridization with SPR sensor of the present invention. The solution is flowed through the surface of SPR sensor with time in the following manner. 0~90 min: Mobile phase (20 mM phosphate buffer solution (PBS, pH 7.7) containing 300 mM of sodium chloride, 1 mM of EDTA and 100 mM of urea); 90~300 min: Mobile phase containing 1  $\mu$ M of DNA which sequence is SEQ ID NO: 25'-CATCCGTGTGGTAAC-3'; 300 min~: Mobile phase; and

[0027] Figure 7B shows detection of DNA hybridization with conventional SPR sensor. The solution is flowed through the surface of SPR sensor with time in the

following manner. 0~30 min: Mobile phase (20 mM phosphate buffer solution (PBS, pH 7.7) containing 300 mM of sodium chloride, 1 mM of EDTA and 100 mM of urea); 30~135 min: Mobile phase containing 1  $\mu$ M of DNA which sequence is SEQ ID NO: 25<sup>2</sup>-CATCCGTGTGGTAAC-3<sup>2</sup>; 135 min~: Mobile phase.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

**[0028]** Several preferred embodiments of the present invention are described in detail below with reference to the drawings annexed, in which Figures 1, 2, and 6 are not drawn to scale.

Example 1: Surface plasmon resonance (SPR) sensor with high sensitivity

**[0029]** As indicated in Figure 1, a SPR sensor comprises an incident light source (1), a prism (2), a metallic layer (3), a layer of metallic nanoparticle layer (4), and a light detector (5).

**[0030]** The metallic layer (3) having a thickness of about 50 nm is formed on a surface of the prism (2) by way of for example RF magnetron sputtering method for precisely controlling the film thickness thereof, or alternatively, by co-sputtering or vapor-plating method generally employed to form metallic films. The material adopted for the metallic layer (3) is gold or silver.

**[0031]** The layer of metallic nanoparticle (4) having a thickness of 1-50 nm is formed on the metallic layer (3) by way of RF magnetron sputtering method using dielectric substance and the metal for creating metallic nanoparticles as the target for being mixedly deposited on the metallic layer (3) to form the metallic nanoparticle layer. Alternatively, the solution of a dielectric substance (usually a polymer) containing metallic nanoparticles is applied by spin coating method to form a film on the metallic layer (3). The material adopted for the metallic nanoparticle layer (4) is gold or silver or platinum, and the diameter of the grains is 1-50 nm approximately.

**[0032]** With regard to the incident light source, a generic laser beam is applicable, or, instead, a laser diode array (6), which radiates multiple laser beams as shown in Figure 2 whose polarized components of light are adjusted by a polarizer (7) and a half-wave plate (8) and a platform of the plate (8) rotating at a constant speed to guide the incident light to shoot on the prism (2) is feasible. In such a mechanism, since different polarized light beams may excite different vibrating or rotating

**Example 6:** Using high-sensitivity SPR sensor of the present invention to detect DNA hybridization

**[0046]** A SPR sensor of the present invention and a conventional SPR sensor are prepared to detect DNA hybridization in the following manner. A SPR sensor (A) is fabricated according to the device of SPR biosensor described in example 5, a conventional SPR sensor (B) is fabricated in the same way but without metal nanoparticle layer.

**[0047]** A 15 ~~mers~~mers of DNA which sequence is SEQ ID NO: 15'-GTTACCACACGGATG-3' is employed as a probe DNA. Fabrication of DNA probe on sensor (A) is carried out in the following manner. A solution which is prepared by mixing equivalent volume of concentrated sulfonic acid and 30% of hydrogen peroxide is dropped and stayed onto the surface of metallic nanoparticle layer of sensor (A) for 2 minutes at room temperature. The treated surface of sensor (A) is rinsed with de-ionized water, ethanol and dried with pure nitrogen. A Solution containing (3-aminopropyl) triethoxysilane (APTES) and ethanol is dropped onto the treated surface of sensor (A), and then the surface is rinsed with ethanol and dried with pure nitrogen. The sensor is immersed into a solution containing 1  $\mu$ M of DNA probe, 1mg/ml of N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC hydrochloride) and 40 mM of 2-(N-Morpholino) ethanesulfonic acid (MES) at room temperature overnight to immobilize the DNA probe onto the surface of sensor (A). The surface of sensor after immobilization of DNA probe is rinsed with de-ionized water and immersed into methanol overnight. The sensor (A) with DNA probe is rinsed with de-ionized water and stored in the MES buffer at 4°C prior to use.

**[0048]** Fabrication of DNA probe on sensor (B) is carried out by immersing the surface of metallic layer of sensor (B) into a solution containing 1  $\mu$ M of DNA probe, 1mg/ml of EDC hydrochloride and 40 mM of MES buffer. The sensor is immersed into methanol to replace the unreacted EDC molecules after fabrication of DNA probe. The sensor is rinsed with de-ionized water and stored in the MES buffer at 4°C prior to use.

**[0049]** Both the two SPR sensors (A) and (B) with DNA probe are applied to detect a dynamic hybridization between DNA probe and its complementary DNA (cDNA) in the analyte. The mobile phase flowing the surface of sensor is a 20 mM

phosphate buffer solution (PBS, pH 7.7) containing 300 mM of sodium chloride, 1 mM of EDTA, and 100 mM of urea. The mobile phase is pumped through the surface of the sensor at a flow rate of 50  $\mu\text{m}/\text{min}$  and the temperature is controlled at 27°C. After the sensing system is stable, the sample solution containing mobile phase and 1  $\mu\text{m}$  of DNA which sequence is SEQ ID NO: 25'-CATCCGTGTGGTAAC-3' is flowed through the surface of sensor. The mobile phase without DNA is reflowed through the surface of sensor to remove the DNA molecules binding to probe weekly until the ascending trend of the SPR angle shift is tardy or even no SPR angle shift is observed.

**[0050]** The result for detection of DNA hybridization with the two SPR sensor is illustrated in Figure 7. A large SPR angle shift is observed in Figure 7A to detect DNA hybridization with SPR sensor (A), it is about 10 times of the SPR angle shift of conventional SPR sensor (B) (shown in Figure 7B). The result shows that the sensitivity of detection using the SPR sensor of the present invention can be advanced one order in sensing biomolecules.